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Effect on *Chlamydia trachomatis* infection of the murine genital tract of adoptive transfer of congenic immune cells or specific antibody

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Summary. Groups of progesterone-treated female CBA/nu mice were adoptively transferred with immune spleen cells or pooled antisera from congenic immunocompetent CBA donors that had been infected with a 'fast', human strain (SA-2f) of *Chlamydia trachomatis.* The spleen cells were given either intravenously (6.3×10^7 cells) or intraperitoneally (9.5×10^7 cells), and the antiserum (antibody titre 1:4096) was given intravenously. Strain SA-2f was introduced into the uterine cavity of these mice approximately 3 h after cell or antiserum transfer; antiserum was given also at intervals up to 23 days later. Untreated mice serving as controls were inoculated with chlamydiae in the same way. Subsequent recovery of chlamydiae from mice in the various groups indicated that transfer of cells or antiserum had not abrogated the chlamydial infections, despite high titres of chlamydial IgG antibody in the sera of all the recipient mice. These results confirm our earlier findings but are unlike those of some other investigators working with different mouse model systems. It seems that there are differences between systemic/respiratory immune mechanisms and those which operate locally in the uterus, which may be regarded as an immunologically privileged site.

Keywords: *Chlamydia trachomatis*, murine genital tract, immune transfer

Chlamydia trachomatis organisms cause a variety of urogenital-tract diseases including non-gonococcal urethritis in men and 'non-specific' cervicitis and acute salpingitis in women which may lead to infertility (Taylor-Robinson & Thomas 1980). Acute disease is probably immunologically mediated, since chlamydial organisms did not cause extensive epithelial cell damage or loss of ciliary activity in organ cultures of Fallopian tubes (Hutchinson *et al.* 1979). The development of a mouse model of chlamydial genital-tract infection has provided much of the basic information that is needed before any logical approach to immunization against chlamydial infection of the human genital-tract can be considered. For example, although larger numbers of chlamydiae were recovered from the genital tract of infected female nude mice than from infected immunocompetent mice, they were cleared from both groups after about the same time (Tuffrey *et al.* 1982). Thus, T lymphocytes or T lymphocyte-dependent antibody would appear to have little effect on the course of this chlamydial infection of the murine genital tract. However, chlamydiae were cleared significantly more quickly from the genital tract of previously infected immunocompetent mice after rechallenge than from previously unin-

fected mice, although initial susceptibility to rechallenge was not affected (Tuffrey *et al.* 1984). This 'immune' effect was again shown to be independent of pre-existing antibody, but must, in some way, have been a result of factors 'memorized' from the previous infection. Since the presence or absence of T lymphocytes alone had been shown to have little effect on the course of infection, it was suggested that perhaps sensitized T lymphocytes in the previously immunized mice had in some way modified the hosts' normal macrophage functions conferring special bactericidal activity on them. The following transfer experiments were carried out to further clarify the role of sensitized cells and antibody in the control of chlamydial genital-tract infection.

Materials and methods

Mice. Adult female inbred strain CBA mice and congenic CBA/nu mice which had been bred and maintained in the specific-pathogen-free unit at the Clinical Research Centre were used. The CBA/nu mice were kept, after inoculation, in an isolator which had filtered inlet air, and were removed only briefly for experimental procedures. This minimized exposure to flora from the conventional holding room.

C. trachomatis strain. The 'fast' egg-killing human strain of *C. trachomatis*, designated SA-2f, was used. This is a genital strain which has been identified as an LGV-2 serotype.

Infection of mice. Four groups of CBA/nu mice were given 2.5 mg of the progesterone preparation Depo-provera subcutaneously a week before and at the time of inoculation of strain SA-2f, in order to help to initiate infection (Tuffrey & Taylor-Robinson 1981). Six mice served as a control group, while antiserum was passively transferred to a group of 10 mice. Two further groups of six mice each were used for adoptive transfer of different doses of immune spleen cells. All four groups were inoculated with 1×10^4 inclusion-forming units (ifu) of strain SA-2f, which was introduced directly into the uterine cavity through a 30-gauge needle, approximately 3 h after treatment with cells or pooled antisera. To do this, the mice were anaesthetized with pentobarbitone given intraperitoneally (0.01 ml of Sagatal/g body weight), and the uterus was exposed through a small lateral incision over the ovarian fat pad. The peritoneum was closed with a fine silk suture and the skin with a surgical clip.

Spleen cell transfer. Nineteen CBA donor mice, which had received three or four inoculations of SA-2f, were used. The first one or two inoculations were via the genital route and then two doses were given intraperitoneally, 11 days apart, approximately 10 months later. The mice were treated with rifampicin (5 mg/kg body weight) on days 9, 7 and 5 prior to killing to ensure that the donor cells were free from viable chlamydiae; this was shown subsequently to be so.

The spleens were removed aseptically 15 days after the last inoculation and were minced and homogenized gently in a ground-glass homogenizer. The debris was allowed to settle and the cells were removed. They were washed once and resuspended in Eagles MEM containing 10% fetal calf serum, vancomycin (100 μg/ml) and streptomycin (50 μg/ml). Viability of the spleen cell suspension by trypan blue exclusion was >85%, and it contained 3.1×10^8 viable cells/ml. The pooled sera from these immunized donor mice had an IgG antibody titre, measured by a micro-immunofluorescence test (Thomas *et al.* 1976), of 1:2048. The 'immune' donor spleen cells were inoculated into two groups of progesterone-treated congenic CBA/nu recipient mice either intraperitoneally (9.5×10^7 cells per mouse) or intravenously (6.3×10^7 cells per mouse) approximately 3 h before inoculation of strain SA-2f.

Serum transfer. A pool of sera, different from

that mentioned above, from CBA mice immunized as a result of several previous inoculations of strain SA-2f, was used for passive transfer of antibody. The chlamydial IgG antibody titre as measured by micro-immunofluorescence was 1:4096. A volume of 0·1 ml of the serum was given intravenously to each of 12 progesterone-treated congenic CBA/nu mice approximately 3 h before intrauterine inoculation of chlamydiae. Additional aliquots of the antiserum were administered through a tail vein on days 3, 7, 9, 15 and 23 after inoculation of strain SA-2f.

Detection of chlamydiae. The vagina of each mouse was swabbed with a sterile cotton-wool-tipped nasopharyngeal swab (Medical Wire and Equipment Company Ltd) at about weekly intervals from the fifth day to the 118th day after inoculation. Each swab was agitated and expressed in 1 ml of cold sucrose–phosphate transport medium (2SP). The specimens were stored in liquid nitrogen until inoculation into cycloheximide-treated McCoy cell cultures for the isolation of chlamydiae, as described previously (Thomas *et al.* 1977).

Antibody studies. The titres of IgG antibody to *C. trachomatis* in all groups of mice were measured throughout the experiments by a micro-immunofluorescence technique in which a fluorescein-labelled rabbit anti-mouse IgG conjugate was used (Thomas *et al.* 1976). The mice were bled from the tail for individual serum samples on days 8 and 35 after inoculation of chlamydiae. The mice which had received antiserum passively were tested also 2 days after the first injection of antiserum. The mice which had received cells were bled additionally 85 days after inoculation of chlamydiae.

Results

Isolation of chlamydiae

The recovery of chlamydiae from mice in the various groups is shown in Table 1. Chlamydiae were not isolated from mice in the control group, that is from those which had not received antiserum or cells, after day 61; mice in this group started to die from day 75, probably due to colonization with the natural bacterial flora expected in nude animals of this age exposed to conventional conditions. Chlamydiae were isolated at some time from all the mice which received congenic immunocompetent cells and these animals survived longer, enabling chlamydiae to be isolated from a few mice up to day 111. Indeed, there was no evidence that the transfer of cells had abrogated the chlamydial infection, although only a few chlamydiae were isolated from days 40 to 57. Unfortunately, the mice which had received antiserum were killed just before day 57. However, all of the nine mice in this group were infected on day 47, at which time a maximum of only two-thirds of the mice in the other groups were chlamydia-positive.

Antibody responses

The titres of chlamydial IgG antibody in serial sera from each mouse are presented in Table 2. The nude mice in the control group were unable to mount an antibody response as indicated by the results of tests on days 8 and 35 after inoculation of chlamydiae.

Transfer of immune spleen cells from congenic immunocompetent mice enabled the recipient nude mice to develop high titres of antibody 8 days after inoculation of chlamydiae, titres which increased and persisted up to day 89, the last day of testing.

Passive transfer of antichlamydial serum (titre of 1:4096) induced titres of up to 1:64 in the recipient nude mice 2 days later. Further administration of antiserum on the third and seventh days increased the titres up to 1:512, as indicated by tests on the eighth day. On day 35, 12 days after the last inoculation of antiserum, antibody was persisting although the titres were much reduced. These various results indicated that

Table 1. Isolation of chlamydiae from progesterone-treated CBA/nu mice treated with immune spleen cells or antiserum

Previous treatment	No. in group	No. of mice from which chlamydiae were isolated on indicated day after inoculation																
		5	13	19	26	33	40	47	57	61	68	75	82	89	96	104	111	118
None	6	5 (2–105)	6 (21–2436)	5 (3–245)	6 (15–264)	5 (*)	5 (6–395)	4 (24–129)	2 (16.20)	2 (16.214)	0	0 1D†	0	0 2D	0	0	0 3D	0 5D
Approx 6.3×10^7 immune spleen cells i.v. 3h before inoculation	6	6 (19–384)	6 (1–117)	5 (26–128)	5 (22–76)	5 (5–20)	5 (1–7)	4 (1–5)	2 (3,10)	5 (1–67)	4 (5–136)	2 (1,21)	0	2 (1,3)	0	0	0	0
Approx 9.5×10^7 immune spleen cells i.p. 3h before inoculation	6	5 (3–39)	4 (4–11)	5 (1–86)	4 (1–42)	4 (1–62)	4 (1–7)	2 (1,4)	2 (2,3)	5 (2–126)	5 (1–35)	5 (2–17)	3 (2–14)	0	2 (4.25)	1 (16)	1 (28)	0
0·1 ml antiserum i.v. on days 0, 3, 7, 9, 15 and 23	9	9 (2–368)	8 (14–712)	9 (9–148)	9 (10–237)	8 (7–276)	9 (3–200)	9 (1–148)	Accidently killed									

* Cell monolayers inadequate for accurate inclusion counts.
†D = no. of animals dead.
Ranges of number of inclusions per McCoy cell monolayer are shown in parentheses.

Table 2. IgG chlamydial antibody titres of sera from progesterone-treated CBA/nu mice inoculated with strain SA-2f 3 h after transfer of immune cells or antiserum

Previous treatment	Mouse No.	Reciprocal of serum antibody titre on indicated day after inoculation			
		2	8	35	89
Control CBA/nu —no treatment	1	nt	0	0	nt
	2	nt	0	0	nt
	3	nt	0	0	nt
	4	nt	0	0	nt
	5	nt	0	0	nt
	6	nt	0	0	nt
Approx. 6.3×10^7 immune spleen cells i.v.	1	nt	16	>2048	2048
	2	nt	512	>2048	>2048
	3	nt	16	>2048	>2048
	4	nt	64	>2048	>2048
	5	nt	128	1024	1024
	6	nt	256	512	1024
Approx. 9.5×10^7 immune spleen cells i.p.	1	nt	512	512	1024
	2	nt	256	1024	1024
	3	nt	64	2048	>2048
	4	nt	128	>2048	>2048
	5	nt	128	>2048	>2048
	6	nt	256	>2048	>2048
0.1 ml antiserum i.v. on days 0, 3, 7, 9, 15 and 23	1	64	512	16	nt
	2	64	64	4	nt
	3	64	128	0	nt
	4	64	128	4	nt
	5	64	256	8	nt
	6	32	128	8	nt
	7	16	64	0	nt
	8	8	512	8	nt
	9	32	256	2	nt

nt, not tested.

serum antibody had no effect in ameliorating the genital-tract infection.

Discussion

The results of these experiments show that adoptive transfer of immune cells or antiserum from congenic immunocompetent donor mice to nude recipients did not protect them from subsequent chlamydial infection. Relatively high titres of antibody were found in the sera of mice to which antiserum had been passively transferred, certainly for the first 10 days, but the susceptibility of the mice to the original infection was not influenced by the transferred serum. Nor did it shorten the course of the infection. In fact, although it is not certain, it seems that infection might have persisted for a longer period of time in the mice that received the antiserum.

Although the duration of infection was not decreased by administration of immune

cells, the numbers of chlamydiae isolated, as indicated by the inclusion counts, were less than those recovered from the control mice. This finding was in accordance with the results of our earlier experiments in which chlamydiae were isolated in larger numbers from nude mice than from their immunocompetent controls (Tuffrey *et al.* 1982). It was apparent that the transferred cells enabled the recipient chimaeras to survive longer than their nude counterparts and to produce higher titres of IgG antibody. Nevertheless, the mice which had both cell mediated immunity and high titres of antibody were not protected.

These results are unlike those of some other investigators working with different mouse model systems. For example, Kuo *et al.* 1982, who inoculated trachoma and lymphogranuloma venereum biotypes of *C. trachomatis* intravenously to induce systemic infection in mice, were able to show that adoptive transfer of cells, but not antiserum, could confer immunity. On the other hand, Williams *et al.* (1982), who used the mouse pneumonitis (MoPn) agent to infect the lungs of nude and immunocompetent mice, demonstrated a role for antibody in defence against the agent. Thus, passive transfer of immune serum delayed the death of both groups of mice compared to controls given normal serum. The protection of nude recipient mice was transient and lasted only while the antiserum was given, while it was longer lasting in the immunocompetent recipients probably because they were able to produce their own antibody as well. Spleen cells from immune donors were also able to protect nude recipient mice against lung infection by the MoPn agent, but this could not be related to cell-mediated immunity alone because the nude recipients also produced antibody as a result of the transfer of the cells (Williams *et al.* 1982). These workers went on to show that this protection correlated better with antigen-specific lymphocyte transformation than with antibody production (Williams *et al.* 1984) and concluded that T cell-dependent cellular immunity is important in defence against pneumonia caused by the MoPn agent.

The demonstration in the studies described here that passive transfer of antibody, or cells which were also capable of producing antibody, did not protect recipient mice from a genital infection with *C. trachomatis* supports our earlier findings (Tuffrey *et al.* 1984). They are in contrast to the results of other workers mentioned above and suggest that there are differences between systemic/respiratory immune mechanisms and those which operate locally in the uterus, which may be regarded as an immunologically privileged site. Furthermore, our observations suggest that control of human chlamydial genital infections through immunization is not a promising approach. However, whether an immunization schedule in the mouse model can protect against disease, such as chlamydial induced salpingitis, rather than against infection is worth exploring.

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